Extracting a Plant Dye (Saffran Dye) Used as Antibiotic to Treat Bacterial Diseases

Dhulfiqar Ali Hammed, Mohammed Hadi Shinen, Abbas Ibrahim Obayes

Department of Physics, College of Science, University of Babylon, Babylon, Hilla, Iraq

e.Mail:address: sci486.zuelfakar.ali@uobabylon.edu.iq

Abstract :

The process of extracting saffron dye, the main derivative of the saffron plant, is carried out extensively and has been acknowledged for its therapeutic properties. Consequently, the objective of this investigation is to develop a novel, superior plant dye and explore its optical characteristics, structural features, and morphological composition, as well as its antibacterial properties, when extracted from the plant (referred to as saffron dye). The determination of the dye's absorbance and quality was carried out through ultraviolet (UV) analysis, a spectrophotometric technique that quantifies dye extracts at three distinct maximum wavelengths, specifically 257 nm (Picrocins), 330 nm (safranal), and 440 nm (Crocins). Additionally, the infrared absorption and emission spectrum were evaluated utilizing Fourier-transform infrared (FTIR) spectroscopy, this facilitated the identification of the initial peaks at which the saffron dye underwent absorption, and made it possible to analyze the vibrational behavior of the bonds present at these peaks. The research included examining the surface morphology using atomic force microscopy (AFM), which allowed the surface roughness of the vegetable dye to be determined. Also, the vegetable dye purity was verified. The surface morphology analysis was conducted using both an electron microscope and a scanning electron microscope, owing to their exceptional precision. Consequently, the plant dye exhibited an oval surface appearance characterized by the presence of oval peaks adorned with short filaments, some of which displayed regularity while others were irregular. The elemental composition of the plant dye was also ascertained using energy-dispersive X-ray spectroscopy (EDX), with varying proportions being observed. Additionally, the antibacterial properties of the plant extract were evaluated, demonstrating bactericidal effects against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas,* and *Escherichia coli*. Notably, the bactericidal effects were observed against both positive and negative bacteria. As a result, the petal extracts of Crocus sativus L. can be regarded as valuable natural sources of antibacterial agents.

Keywords: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas, and Escherichia coli*, Saffran dye.

1. Introduction

Saffron is exceedingly precious, composed of the desiccated pistils of Crocus sativus L., exceptional, unparalleled, irreplaceable, and exceedingly lucrative both as a piquant and therapeutically beneficial botanical specimen[1]. Many plants are medicinal, and some have strong antimicrobial effects. The antimicrobial activity of certain dyes is potent due to the presence of safranal and crocin compounds[2]. saffron has been used in traditional medicine and now is the focus of biomedical research [3][4]. The properties mentioned above are intrinsically linked to the components of picrocrocin, safranal, and crocins. Picrocrocin serves as the glycoside precursor of safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), which is the most abundant volatile compound contributing to the aroma of this spice. On the other hand, crocetin esters joined with glucose, gentiobiose, neapolitanose, or triglucose sugar molecules make up crocins. The reason for the yellow hue of saffron is these water-soluble carotenoids [5]. Saffron (Crocus sativus L.) was a traditional medicinal herb used for ages in Greece, Iran, Spain, China, India, and Italy[6]. Strong antioxidant qualities are well known about saffron (Crocus sativus L.). Safranal, crocetin and crocin are the three primary active components of saffron. Moreover, saffron has over 150 volatile molecules that give it its scent in addition to a number of non-volatile elements including polysaccharides and

carotenoids including zeaxanthin, lycopene, and beta-carotene. Because oxidative stress is so important in many disorders, the possible therapeutic use of saffron supplements has drawn a lot of interest. The eighth September. Traditional medicine has used saffron and its derivatives for its sedative, analgesic, antiemetic, antispasmodic, and antidepressant properties. Additionally utilized as a hypnotizing and anticonvulsant medication is saffron. Among the many ailments that saffron has been tried to treat is irregular menstruation [7] [8]. Conventional knowledge holds that saffron may be used as an emmenagogue for amenorrhea and to induce menstruation. Saffron may relieve spasmodic symptoms and trigger miscarriage or abortion. Because saffron is stimulating, mental health may be enhanced. In Ayurvedic, ancient Indian medicine, saffron is regarded as an adaptogen that helps the body resist stresses including anxiety, fatigue, and trauma [9].

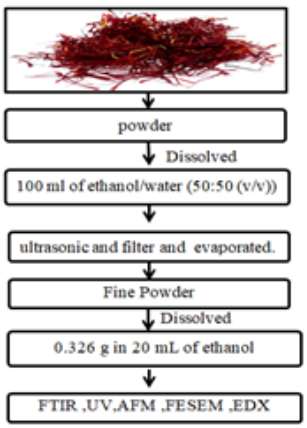
The substantial antioxidant properties of saffron (Crocus sativus L.) have generated interest in its potential as a medicinal supplement for a range of health conditions since oxidative stress has a major influence on many illnesses. Saffron is used traditionally in medicine for its antidepressant, antiemetic, antispasmodic, and sedative effects[7]. Its anticonvulsant and hypnotic qualities have also been used to a number of ailments, including irregular menstruation. Saffron is thought in traditional medicine to be a menopausal antiseptic and to promote menstruation. Additionally recognized to help and induce miscarriage, the herb also alleviates spastic symptoms. Besides, saffron's antidepressant qualities are well recognized to improve mental wellness. Saffron is an adaptogen in ancient Indian medicine, particularly in Ayurveda, that strengthens the body's resistance to stresses like trauma, anxiety, and exhaustion[9].

The phenolic composition and bactericidal properties of hydroethanolic extracts from Crocus sativus L. were investigated recently by Nadia Naim and companions. 27 phenolic compounds in all were identified in the petal extracts of Crocus sativus L., mostly flavonoids produced from kaempferol, quercetin, isorhamnetin, and myricetin. These petal extracts had bacteriostatic effects against Salmonella typhimurium and Escherichia coli in addition to bactericidal activity against Staphylococcus aureus and Listeria monocytogenes when they were hydroethanolic. Petal extracts from Crocus sativus L. may thus be regarded as important sources of natural antibacterial agents for use in the food and pharmaceutical sectors[10]. Studying the efficacy of saffron (Crocus sativus L.) in premenstrual syndrome, labor, delivery, and menopause was Morvarid Irani et al. in 2023. The study found that saffron improved Bishop's score, advanced labor, decreased exhaustion and pain intensity during labor, and promoted episiotomy healing. It also was shown to help with psychological problems and mood swings related to menopause and PMS. Two studies suggested that saffron may help with postpartum depression [9]. The aim of study a lot of research has been done on the process of extracting the dye, its composition, its structural characteristics, and its impact on both positive and negative bacteria on the skin when stored at room temperature. This is because medicinal plants, particularly saffron, are becoming more and more popular.

2. Materials and Methods

2.1 Properties and uses of saffron

The presence of 14–16% water, 11–13% nitrogenous materials, 12–15% sugars, 41–44% soluble extracts, 0.6–0.9% essential oil, 4–5% fiber, and 4–6% ashes are characteristics of the stigmas. The saffron dye was extracted using a solvent extraction process. For this purpose, 1.0 grams of saffron manufactured from Iranian farms in the South Khorasan Province were dissolved in 100 ml of ethanol/water (50:50 (v/v)), sonicated for 10 minutes in an ultrasonic bath, and filtered. The saffron was dried and prepared for this purpose. After that, the mixture is centrifuged for ten minutes to filter it. To get a concentrated extract, it is then evaporated in a vacuum. Ultimately, the concentrated solution was powdered after being dried at 50 °C in a vacuum oven. We dissolved 0.326 g in 20 mL of ethanol and stirred for half an hour. Fourier Transform Infrared (FTIR) spectrometer spectra and a spectrophotometer equipped with a UV-visible with a wavelength of 445 nm were used to measure the absorbance of each solution. For the solution before as well as after drying and EDX, as illustrated in Figure (1), which shows the block diagram of stages involved in the experiment.



Figure(1): Block diagram of the experimental steps.

3. Results and Discussions

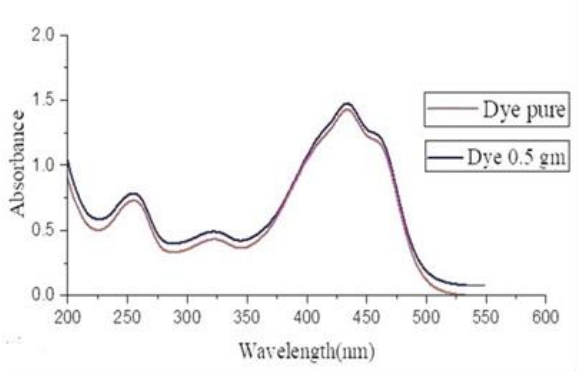
3.1 UV-Vis Spectroscopy

Using a UVIKON 943 double-beam UV-Vis spectrophotometer (Kontron Instruments, Milan, Italy), the analyses were performed according to the guidelines stated in Paragraph 14 of the ISO 3632-2:2010 standard The properties associated with this solution's absorption At room temperature for the liquid dye twice including one at the process with 0.5 gm dissolving and the other for the powder extraction from the solvent for the Saffran for the wavelength (200-600) nm was measured. It is evident from the illustration that both spectra contain the same peaks and are congruent. Fig (2) illustrates the variation of absorbance opticals with wavelength for both methods. Figure 2 displays the average spectrum for each plant sample. Fig 2 illustrates that the saffron samples' greatest absorbance was seen at 440 nm. Using a (1) cm pathway quartz cell, the spectrophotometric quantification of the stigmas' extracts at three maximum wavelengths—257 nm for flavor strength (picrocrocin), 330 nm for aroma (safranal), and 440 nm for coloring force (crocins)—is the methodology to determine the quality of saffron employing these regulations. [12][11]. The absorption spectra of the crocins span from 250 to 470 nm and exhibit overlaps between the compounds at different wavelengths. This is saffron dye's feature[12][13].

Power of color(coloring force) = 440 nm= crocins

Strength of flavor (bitterness)= 257 nm= (picrocrocin)

Strong smell (aroma)= 330nm = (safranal) [14].



Figure(2): Ilustrates Saffron dye's UV–visible absorption curve.

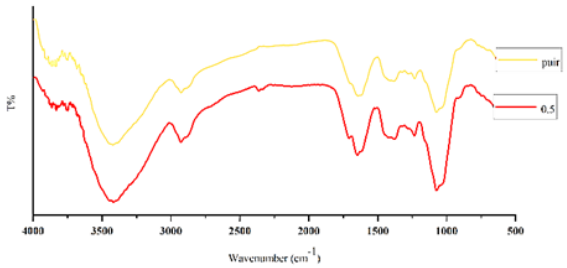
3.2 ATR-FTIR Spectroscopy

To test the pure saffron dye, a tiny amount was taken and put under the tip of a Thermo Electron Corporation, Waltham, Massachusetts, USA, Nicolet 380 FTIR spectrometer. ATR sampling was used to capture a spectrum in transmittance mode, spanning from 500 cm^{-1} to 4000 cm^{-1}. 64 scans were conducted with a resolution of 4 cm^{-1}, and smoothing procedures were performed. Figure(3) illustrates the FTIR Spectroscopy of Saffron dye. Demonstrate that the peaks in the fingerprint region (1073.77–500) cm^{-1} are connected to the component's skeleton and bending vibrations and may be ascribed to several groups, including the C–C, C–O, CH2–, and CH 3-groups. More specifically, high absorption at 1073 cm-1 is indicative of C–O vibration, whereas CH=CH stretching vibration is shown at 582.28 cm^{-1}[15][16].

In this particular instance, the interval ranging from 500 to 1500 cm^{-1} exhibits a striking similarity to the corresponding spectral attributes of a material derived from lignocellulosic biomass[17]. peaks at 1647.27 cm^{-1} correspond to carbonyl >C=O groups (esters) or -C=O stretching vibrations[15][16]. Figure 3 depicts the presence of both saffron and saffron pure dye exhibiting extensive absorption bands without distinct peaks but rather overlapping ones, indicating the presence of numerous constituents[18][19].

Our discoveries align with other research discoveries. [18][19]. Saffron exhibits a highly intricate composition, akin to most naturally occurring substances, whereby the FT-IR spectra for its constituents, namely carotenoids, polysaccharides, and fatty acids, overlap. Within saffron, one can find glycosyl esters of crocetin,

specifically crocins (carotenoids), safranal (monoterpene aldehyde), and picrocrocin (glycoside of safranal). This fact was substantiated through the identification of peaks obtained from the UV-Vis Spectroscopy analysis of the extracted saffron dye, as depicted in Fig (2).

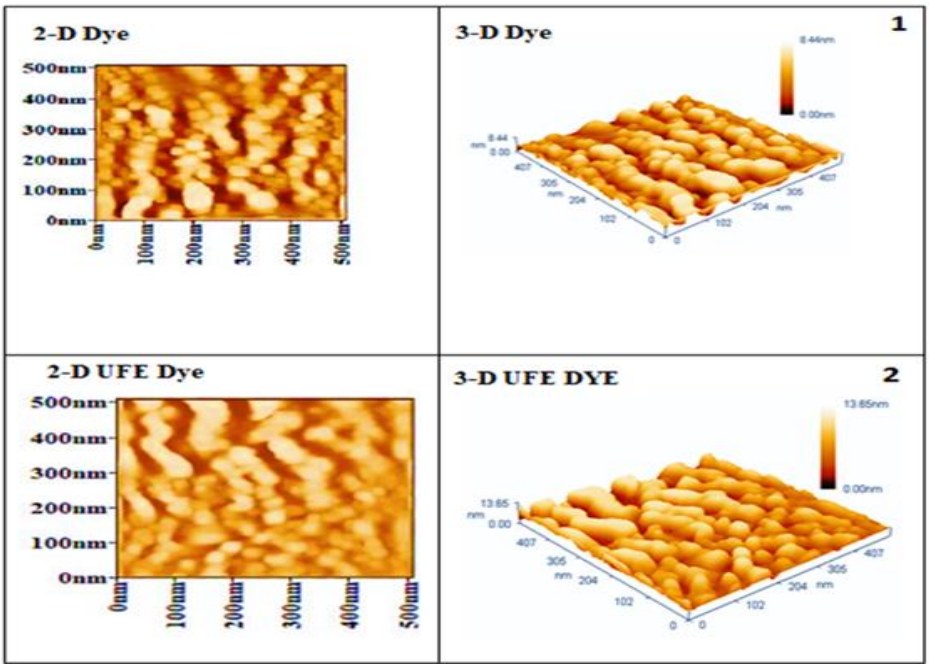


Figure(3): FTIR Spectroscopy illustrates of Saffron dye.

3.4 Atomic Force Microscopy (AFM)

It illustrates the surface topography of the dye that was deposited by Casting the dye onto the glass surfaces. Extensive AFM analysis for the simple of saffran dye and saffrandye (UFE) was conducted using SPM-AFM contact mode to describe the morphology and other surface parameters. Fig (4:1) shows a 2D and 3D images that show saffran dye (UFE) after an ultrasonic bath, filter, and evaporation while Fig (4:2) saffran dye Immediately after dissolving the saffron with water, and ethanol (50:50). By visual evaluation of the topographic images, it can be shown that sufficient homogenization has been obtained for each sample through the dye extraction process. The main action was to evaluate the homogeneity of the investigated samples made from the saffron plant using AFM of the thin-film surfaces formed by the casting method. The surface roughness average for the samples is used by atomic force microscopy. The average roughness of the dye extracted through filtration was (2.41 nm) and the average roughness of the dye extracted by evaporating and dissolving the filtrate was (1.61 nm). This leads to high pure dye and stronger through the ability to eliminate smaller components, such as picrocrocin lycopene, permitting main color component of saffron) crocin (to remain the only dye component. In this way, filtered saffron is more pure and rich in crocin, that acts as direct dye[19].

the dehydration of the dye leads to the undesired structural collapse detachment and creates structural porous discrimination that structures uniform tissue conjunctive for the surface stigma[20]. Also, during the drying process of the thin film at room temperature, picrocrocin loses sugar residues, which are rough, and this is the reason why the roughness of the films is low[12].



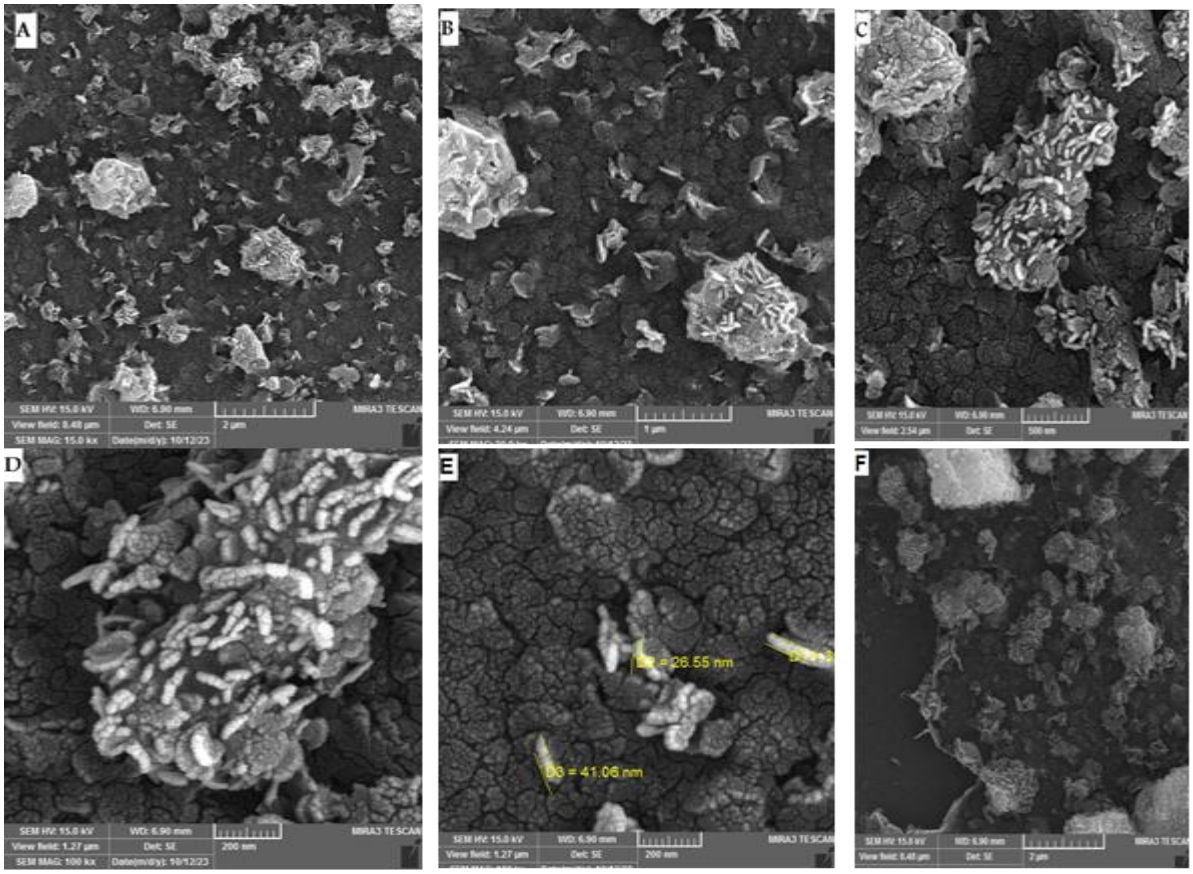
Figure(4):2D and 3D AFM images of the produced saffran dye (UFE), (2) 2D and 3D AFM images of the produced saffran dye Immediately after dissolving the saffron with water and ethanol (50:50).

3.5 Scanning Electron Microscopy (SEM)

The scanning electron microscopy (SEM) method may be used to demonstrate the dispersion of saffron dyes because to its 3-D representation, high resolution, and ability to produce flawless pictures. The saffron dye exhibits a far more varied and diverse morphological appearance, as seen in Figure (5). When (5-A) Some of the samples consisted of almost identical irregular shards as those obtained in the thread samples, as shown in Fig(5-B). The surface is elliptical and has oval peaks adorned with short filaments, some of which exhibit regularity while others have irregular shapes. These peaks are spaced at short intervals, as seen in the picture (C, D). The particles have dimensions ranging from 26 to 42 nanometers, as shown in Figure E. Short threads do not contain any impurities in these colors. Meanwhile, some shards were oddly formed in other cases. Fig (5-C) exhibited a higher prevalence of irregular particles with indeterminate size and form in some instances.In certain cases, one may see the existence of pollen granules, shown as Fig(E), which are spherical/ovoid particles with a varied range of sizes. The look may be ascribed to the pollutants stemming from the growing and processing phases[21].

Nevertheless, scanning electron microscopy (SEM) pictures revealed the presence of extensive fissures and irregular particles with indentations, as seen in figure (f). The significant decrease in the quantity of pollen grains could be attributed to the loss of conjunctive tissue in cellular structures, leading to extreme deformations. On the other hand, stigmas maintained consistent structures, as confirmed by other studies. In this instance, the pollen grains were ensnared in the papillae, maintaining their spherical form and preventing separation in Fig(5-C).

The process of drying the dye results in the evaporation of the solvent, which in turn creates a porous structure inside the inner core of the dye. An alternative perspective suggests that an outer layer with a glass-like texture is formed as a result of the quick reduction in moisture content, which prevents the collapse and contraction of the plant stigma cells[20].



Figure(5): FESEM micrographs of selected whole dye:
A– Saffron dye showed a morphological appearance.
C, D–The surface, peaks are covered with short threads and short distances.
E –Dimensions of particle dye.
F–The surface cracks structure and irregular particles with indentation.

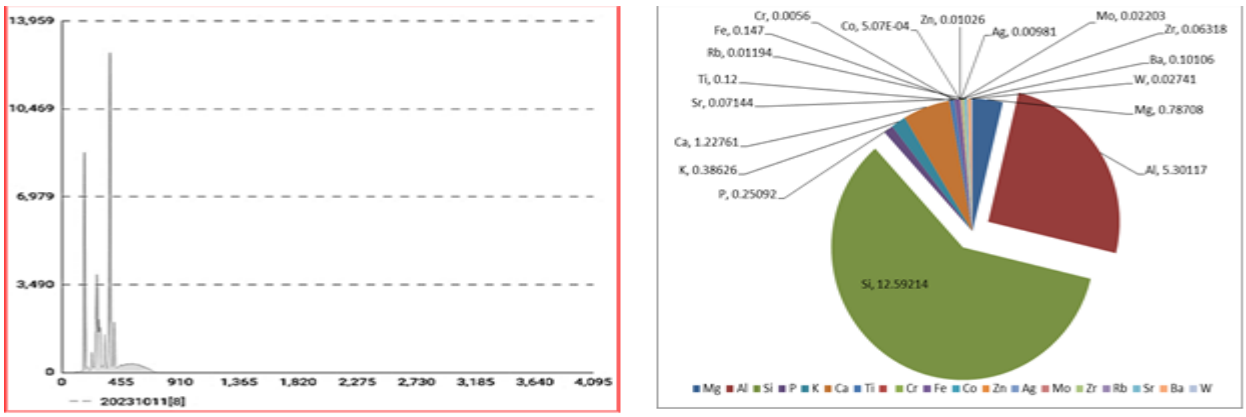
3.6 Energy Dispersive X-ray (EDX) microanalysis

In order to validate the findings from the UV and FTIR studies, we conducted microscopic examinations using SEM and quantitative analyses using EDX to identify the constituents present in this dye. Figures (6) show the Energy Dispersive X-ray (EDX) analysis results for the Dye.
Energy-dispersive X-ray (EDX) is a method of analysis that is used to determine the elemental composition or chemical makeup of a given sample. Figure (6) depicts the EDX data for saffran dye generated from a natural source, namely the saffron plant. This dye is known for its exceptional sensitivity in identifying different components in tissues. EDX microanalysis is utilized extensively in several biological research fields. The table displays the elemental makeup of saffron dye, represented as a weight percentage of each element's oxide. The dye samples exhibited an existence of many elements in different proportions, which was reflected in the greatest mean concentrations, comprising silicon, iron, calcium, and aluminum.

The sample dye had a significant amount of silicon, which was the most prevalent element. The silicon content exhibited little fluctuation, with values ranging from 12.6 to 0.009. All the other substances being analyzed (chromium, zinc, rubidium, cobalt, molybdenum) were detected at levels below the limit that can be accurately measured, as seen in Figure 10. The elemental makeup of saffron may vary significantly based on several circumstances, such as the origin of the specimen [22] [26]. On the contrary, the samples dye showed a much larger amount of the particles described before, which was also evident in the higher average concentrations of Fe, Ca, Si, and Al. These findings may also be attributed to the abundance of pollen grains, which have a high concentration of these components. The primary component of saffron color is potassium (K). The quality of saffron is determined by its chemical composition, which imparts a bitter flavor, a pleasing scent, and an appealing yellowish-red color to this spice[23][24].

Table 1. T he elemental composition of the saffron samples is expressed as a percentage by weight of the element's oxide.

Element	Content (%)
Mg	0.79
Al	5.30
Si	12.59
P	0.25
K	0.39
Ca	1.23
Ti	0.12
Cr	0.01
Fe	0.15
Zn	0.01
Ag	0.01
Mo	0.02
Zr	0.06
Rb	0.01
Sr	0.07
Ba	0.10
W	0.03



Figure(6): the EDX diagram of saffron dye.

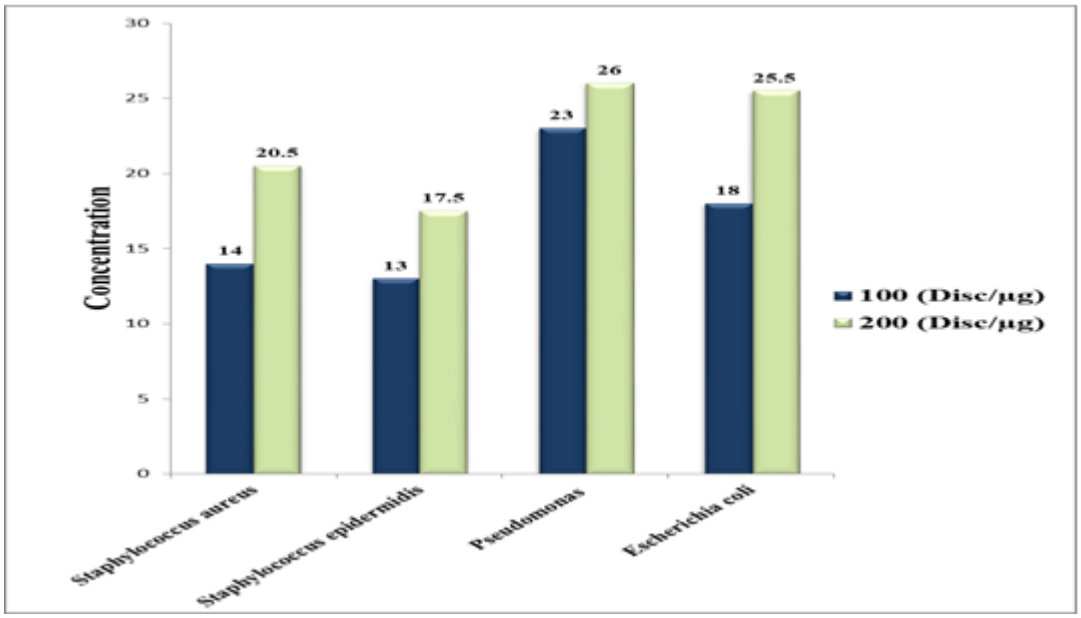
3.7 Antibacterial Efficacy:

a study was conducted to examine the biological effects of compounds derived from the saffron plant (specifically, saffron dye) on pathogenic microorganisms that were obtained from various hospitals in Iraq. The antibacterial properties of the saffron extract (in the form of saffron tincture) were evaluated through a susceptibility test, which involved four types of bacteria commonly found on human skin. The agar-well diffusion method was employed to assess the antibacterial activity of the bioactive compounds extracted from saffron plants against the aforementioned bacteria.[25]. Using cork porers with a diameter of 6 mm, wells were created. Mueller-Hinton Agar was employed as the negative control, and various antibiotics were used as the positive control, depending on the kinds of pathogenic bacteria present. All of the bacterial isolates used in this investigation came from hospitals in the Iraqi city of Hillah. (Table: 2).

Table 2: Types of Bacterial Isolates and their Sources

No.	Bacteria isolate	Type of Bacteria	Type of specimen
1	Staphylococcus aureus	Gram-positive	Burns
2	Staphylococcus epidermis	Gram-positive	skin infections
3	Pseudomonas aeruginosa	Gram-negative	Burns
4	Escherichia coli	Gram-negative	skin infections

The antibacterial activity of compounds extracted saffran plant (saffran dye) using solvent like (Ethanol) against hospital pathogenic bacteria is presented in a Figure(7).



Figure(7): The antibacterial activity of compounds extracted saffran plant (saffran dye)

Saffron dye inhibited the growth of all test bacteria by using the agar diffusion method. The antimicrobial efficacy of saffron dye was observed against both gram-negative and gram-positive bacteria examined in this research. The saffron dye, when used at concentrations of 100 Disc/µg, exhibited distinct inhibitory effects on both types of bacteria, with these concentrations ranging from approximately 13 to 23 percent. however, When the ratio was increased to 200 (Disc/µg), the capacity of the plant extract also increased significantly, as the ratio ranged between (˚26-˚17). The inhibition of bacteria is due to saffron dye, which contains biologically active terpenoids[20]. also, saffron dye contains crocin (gentiobiose ester of crocetin), which has biological activity[23][26]. In addition, through EDX examination of saffron dye, it was found that it contains many important elements that are biologically active and highly effective and most plants can serve as a source of

antimicrobial agents[22]. This is mainly because profusion of the different classes of phenolic compounds [24]. There are many mechanisms used by plant extracts suppress the growth of microbial pathogens are multiple and include cell membrane function disruption, disruption of energy activity, and damage to the cytoplasmic membrane of the bacteria [24][10].

3.8 CONCLUSION

Saffron waste, an agricultural by-product, can be a promising source for producing natural dyes and antibiotics. The vegetable dye was prepared with high quality through Ultraviolet (UV) testing, FTIR, AFM, SEM, and EDX. The prepared dye has three distinct peaks and has the characteristics of color stability across color shades. Through atomic microscope images, he showed the surface topography of the dye that was deposited by throwing the dye on glass surfaces. The results revealed sufficient homogeneity for each sample through the dye extraction process using the average surface roughness of the samples. The average roughness of the dye extracted through filtration is very tiny and evaporation and dissolution of the filtration leads to a highly pure dye and a stronger ability to eliminate smaller components. The natural dye is rich in phenolic compounds and flavonoids and exhibits a high scavenging effect, excellent reducing power, and beta-carotene bleaching prevention, thus it has high antibacterial there for activity as an antibiotic to treat bacterial diseases

References

[1] A. A. D'Archivio, F. Di Donato, M. Foschi, M. A. Maggi, and F. Ruggieri, "Uhplc analysis of saffron (crocus sativus l.): Optimization of separation using chemometrics and detection of minor crocetin esters," *Molecules*, vol. 23, no. 8, 2018, doi: 10.3390/molecules23081851.

[2] A. Çaliş, G. Y. Çelik, and H. Katircioglu, "Antimicrobial effect of natural dyes on some pathogenic bacteria," *African J. Biotechnol.*, vol. 8, no. 2, pp. 291–293, 2009.

[3] J. P. Melnyk, S. Wang, and M. F. Marcone, "Chemical and biological properties of the world ' s most expensive spice : Saffron," *FRIN*, vol. 43, no. 8, pp. 1981–1989, 2010, doi: 10.1016/j.foodres.2010.07.033.

[4] C. Licón, M. Carmona, S. Llorens, M. Isabel, and B. Gonzalo, "Potential Healthy Effects of Saffron Spice (Crocus sativus L . Stigmas) Consumption," 2010.

[5] C. Pintado, A. De Miguel, O. Acevedo, L. Nozal, J. Luis, and R. Rotger, "Bactericidal effect of saffron (Crocus sativus L .) on Salmonella enterica during storage," *Food Control*, vol. 22, no. 3–4, pp. 638–642, 2011, doi: 10.1016/j.foodcont.2010.09.031.

[6] E. Akbari, K. Alami, D. Hossaini, M. L. Nazari, and M. H. Salehi, "Effect of Afghan Saffron (Crocus sativus L .) Aqueous Extract on Withdrawal Signs in Morphine-Dependent Rats," vol. 12, no. 6, pp. 1727–1731, 2020.

[7] D. Abadibavil and S. Dashti, "Investigating the Effect of Saffron on Anxiety , Fatigue , Labor Pain and Vaginal Childbirth : Review Article," vol. 17, no. April, pp. 270–275, 2021.

[8] A. Chang, J. Grigg, and P. Mccluskey, "Critical Reviews in Food Science and Nutrition Efficacy and Safety of Saffron Supplementation : Current Clinical Findings," no. April, pp. 37–41, 2015, doi: 10.1080/10408398.2013.879467.

[9] M. Irani, A. Rahmanian, and N. Soltani, "Efficacy of Saffron (Crocus sativus L.) in Premenstrual Syndrome, Labor, Childbirth, and Menopause: A Systematic Review of Clinical Trials," *Mod. Care J.*, vol. 20, no. 3, 2023, doi: 10.5812/modernc-134533.

[10] N. Naim *et al.*, "Crocus sativus L . Petal Extracts," pp. 1–10, 2023.

[11] L. H. Atyane, J. Molinet, M. A. Serghini, N. Dupuy, and L. E. L. Maimouni, "Influence of drying process on safranal content in the Taliouine Saffron (Morocco): quantification by gas chromatography," vol. 2508, pp. 4597–4603, 2017.

[12] C. R. Avila-sosa, G. V. Nevárez-moorillón, C. E. Ochoa-velasco, A. R. Navarro-cruz, P. Hernández-carranza, and T. S. Cid-pérez, "Chemical Composition and Quality of Saffron," no. c, 2011.

[13] S. Agronomy, "The Effect of Ethanol – Water Mixture on Ultrasonic Extraction of Crocin from Saffron Maede," vol. 9, no. 3, pp. 285–293, 2021, doi: 10.22048/jsat.2021.49280.1146.

[14] S. V. Er, H. Eksi-kocak, H. Yetim, and I. H. Boyaci, "Novel Spectroscopic Method for Determination and Quantification of Saffron Adulteration," *Food Anal. Methods*, 2016, doi: 10.1007/s12161-016-0710-4.

[15] S. A. Ordoudi, M. D. L. Mozos, and M. Z. Tsimidou, "On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR)

spectroscopy and chemometrics," *FOOD Chem.*, vol. 150, no. 2014, pp. 414–421, 2015, doi: 10.1016/j.foodchem.2013.11.014.

[16] E. Anastasaki *et al.*, "Differentiation of saffron from four countries by mid-infrared spectroscopy and multivariate analysis," pp. 571–577, 2010, doi: 10.1007/s00217-009-1197-7.

[17] N. S. P. Sarman, S. Nor, and S. Samsudin, "Computer-assisted analysis of fourier transform infrared (ftir) spectra for characterization of various treated and untreated agriculture biomass," vol. 7, pp. 5367–5380, 2012.

[18] P. A. Tarantilis, A. Beljebbar, M. Manfait, and M. Polissiou, "FT-IR , FT-Raman spectroscopic study of carotenoids from saffron (Crocus sati 6 us L .) and some derivatives," vol. 54, pp. 651–657, 1998.

[19] N. F. N. Panagiotis Naidis , Smaro Lykidou , Magdalini Mischopoulou , Evangelia Vouvoudi, "Study of the dyeing properties of saffron and ultrafiltrated saffron powders, as colourants for natural and synthetic fibres".

[20] F. Kokkinaki and S. A. Ordoudi, "Insights into the FTIR Spectral Fingerprint of Saffron (Crocus sativus L.) Stigmas After Gentle Drying Treatments," *Food Bioprocess Technol.*, vol. 16, no. 12, pp. 3057–3072, 2023, doi: 10.1007/s11947-023-03119-9.

[21] A. Bergomi, V. Comite, L. Santagostini, V. Guglielmi, and P. Fermo, "Determination of Saffron Quality through a Multi-Analytical Approach," *Foods*, vol. 11, no. 20, 2022, doi: 10.3390/foods11203227.

[22] T. Shahi, E. Assadpour, and S. M. Jafari, "Main chemical compounds and pharmacological activities of stigmas and tepals of 'red gold'; saffron," *Trends Food Sci. Technol.*, vol. 58, pp. 69–78, 2016, doi: 10.1016/j.tifs.2016.10.010.

[23] S. A. Babiker and O. K. Yousif, "Chemical composition and quality of camel meat," *Meat Sci.*, vol. 27, no. 4, pp. 283–287, 1990, doi: 10.1016/0309-1740(90)90066-F.

[24] M. Sarfarazi, S. M. Jafari, G. Rajabzadeh, and J. Feizi, "Development of an environmentally-friendly solvent-free extraction of saffron bioactives using subcritical water," *Lwt*, vol. 114, no. June, p. 108428, 2019, doi: 10.1016/j.lwt.2019.108428.

[25] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," *J. Pharm. Anal.*, vol. 6, no. 2, pp. 71–79, 2016, doi: 10.1016/j.jpha.2015.11.005.

[26] M. A. Maggi, S. Bisti, and C. Picco, "Sa ff ron : Chemical Composition and Neuroprotective Activity," pp. 1–15.